In Vitro Migratory Capacity of Adipose-Derived Stem Cells Encapsulated Within An RGD-Modified Chitosan Hydrogel In Response To SDF-1

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Introduction

Biomaterial scaffolds are a proven strategy for improving the retention and survival of cells transplanted to the ischemic heart.[1] Additionally, the co-delivery of chemoattractive factors, such as stromal cell-derived factor-1 (SDF-1), improves stem cell localization and induces revascularization.[2] This work explores the ability of SDF-1 to induce 3-D migration of adipose-derived stem cells (ASCs) through a peptide-modified glycol chitosan-based hydrogel scaffold.

Materials and Methods

Preparation of MGC-GRGDS: N-methacrylate glycol chitosan (MGC) was prepared through reaction of glycol chitosan with glycidyl methacrylate.[3] To functionalize MGC with RGD, *N*-succinimidyl acrylate in dimethylformamide was first added to a GRGDS solution in bicarbonate buffer, at a molar ratio of 7 acrylates per peptide amine terminus. After 4 h, the reaction was dialysed and lyophilized to obtain acrylated-GRGDS peptide. Acrylated-GRGDS was added to an MGC solution in sodium phosphate buffer at a molar ratio of 0.2 acrylate per free amine of MGC. After 12 h at 37 °C, the reaction was dialyzed and lyophilized, yielding peptide-modified MGC (MGC-RGD). Degree of functionalization in the final product was determined by ^{1}H NMR.

ASC Isolation and Culture: ASCs were extracted from epididymal fat pads of 12-week old male Wistar rats and expanded on tissue culture polystyrene.[4] All protocols were approved by the Queen's University Animal Care Committee.

3-D Cell Migration Model: A 3-D cell migration model was developed using a modified Boyden chamber assay. Sterilized MGC-RGD was dissolved in serum-free medium with Irgacure 2959 photoinitiator. In 24-well trans-well inserts (8.0 μm pore size), the MGC-RGD solution was combined with ASCs in serum-free medium to prepare 0.1 mL of 3% w/v MGC-RGD containing $2.5x10^6$ cells/mL. The solution was photo-crosslinked to produce a gel at the interface between the upper and lower chambers. 600 μL of complete medium was added below the gel, and 200 μL of complete medium containing 0 or 50 ng/mL of SDF-1 was added above the gel. The gels were cultured at 37 °C, 5% CO_2, under normoxic (21% O_2) or hypoxic (5% O_2) conditions.

Tracking Cellular Distribution: Immediately after encapsulation, and at days 1, 4, and 7, gels were removed from the trans-well inserts intact and stained with LIVE/DEAD. On the top and bottom of each gel, randomly selected regions representing 15% of the total gel cross sectional area were imaged using a confocal scanning laser microscope at five evenly spaced focal planes extending to a depth of 100 μm into the gel. ImageJ analysis software was used to determine the live cell densities (cells/cm^2) in triplicate gels. Differences in group means were determined via a two-tailed Student's t-test.

Results

MGC-RGD possessed 6% methacrylation and 5% GRGDS functionalization. Following photo-encapsulation, viability was greater than 85% and no significant difference between top and bottom cellular density was observed (data not shown). Cellular densities are presented in Figure 1. At 1 d, all gels exhibited a trend of greater cell densities at the bottom of the gel. This trend

continued in control gels not exposed to SDF-1. Conversely, this trend reversed in gels exposed to SDF-1. These differences between top and bottom cell density were significant ($p<0.05$) at day 7 under normoxic conditions, and at day 4 under hypoxic conditions.

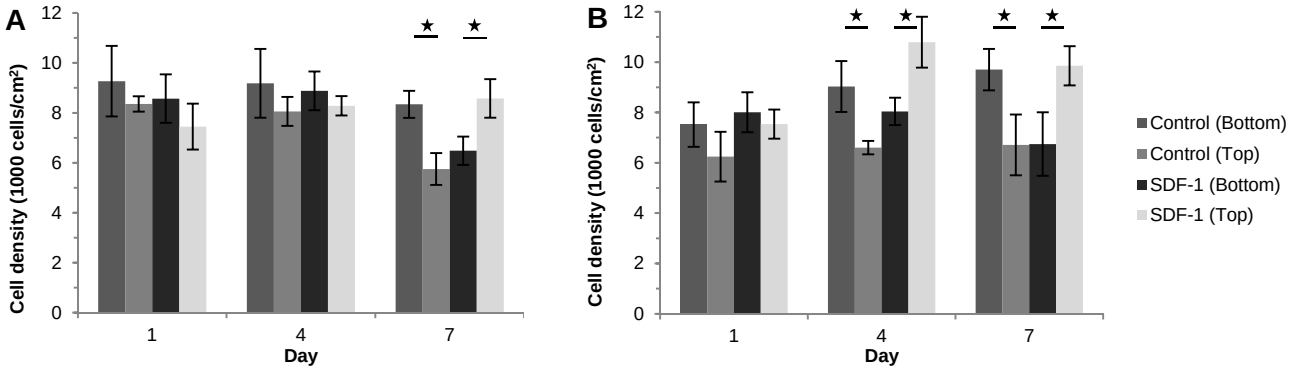

Figure 1: ASC density distributions under A) normoxic and B) hypoxic conditions in control and SDF-1-treated MGC-RGD hydrogels (error bars indicate standard deviation, n=3)

Discussion

SDF-1 is a known chemoattractant of ASCs that is unlikely to have charge interactions with MGC at physiological pH.[2] No significant differences in cell density distribution were observed immediately after encapsulation, indicating the ASCs were initially well-mixed. A trend towards higher densities in the bottom of the gel after 1 d indicate a tendency for ASCs to settle, either before crosslinking, or before the cells have undergone RGD-mediated anchorage. This tendency was maintained in untreated gels, potentially indicating that ASC migration was influenced by gravity or the larger reservoir of media below the gels. In SDF-1 treated gels, the trend had reversed by 7 d and 4 d under normoxic and hypoxic conditions, respectively, demonstrating ASCs were capable of migrating through MGC-RGD towards the apically applied SDF-1. The fact that these effects became significant in less time under hypoxic conditions is consistent with hypoxia induced up-regulation of the SDF-1/CXCR4 chemokine pathway.[2]

These findings suggest that SDF-1 could be utilized to control the localization of ASCs in an MGC-RGD delivery scaffold delivered to the ischemic heart. Whether released from within the scaffold from embedded growth factor-loaded microspheres to improve retention, or externally to promote migration into the surrounding tissue, the co-delivery of a chemokine provides a mechanism to control ASC distribution during the process of myocardial healing. This approach could aid in maintaining localization of the critical number of stem cells required to produce a regenerative milieu of growth factors. Additionally, upregulation of the SDF-1/CXCR4 pathway caused by exposure to endogenous SDF-1 could enhance the regenerative potential of the ASCs, as well as mediate their cytoprotective response to the hypoxic conditions of the ischemic heart.[2]

References

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